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Research Article
Microbial Water Quality and Diazotrophic Bacteria Community in Lake Nasser Khors, Egypt

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Abstract
Management of water quality, control of water pollution and environmental protection necessitated careful routine monitoring, especially Lake Nasser and Khors. Lake Nasser represents a fresh water reservoir and provides more than 95% of the Egyptian fresh water budget. Therefore, this study was carried out to investigate the microbial quality of Lake Nasser Khors (Kalabsha, Wadi-Abyad, Korosko and Tushka) through estimate total bacteria count at 22 and 37°C, total and Faecal Coliforms (FC), Faecal Streptococci (FS) and diazotrophic bacteria. As well as the potential diazotrophs isolates, having >5 n M C_2H_4/culture/hour, were identified by API 20E and API 20NE profiles. The results showed that the microbial load significant differences were attributed to the seasons and the sites. In general, among Khors the downstream Khors (Kalabsha and Wadiabyad) were higher microbial load than the upstream Khors (Toushka and Korosko). The low FC:FS ratio were reported in majority of sample indicating the non-human source of pollution. In addition, diazotrophs were existent in high population densities in all waters sampled along the experimental period. In conclusion, Khors water is generally acceptable for irrigation and was not suitable for drinking purpose without pre-treatment.

Key words: Lake Nasser, khors, microbial water quality, diazotrophs, MPN

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lake Nasser is a reservoir in the course of the Nile river formed as a result of the construction of Aswan High Dam. The lake has many embayments locally called Khors. The total numbers of important Khors reached about 85 Khors. Such as Kalabsha, El-Allaqi, Tushka, El-Sabakha, Singari, Korosko, El-Ramla, Rahma and Wadi-Abyad. Lake Nasser plays a main role in the local and national economy, where it provides more than 95% of the Egyptian fresh water budget. Therefore, many studies covering the quantity and quality of Lake Nasser (Elewa and Azazy, 1986; Rabeh *et al*., 1999; El-Shabrawy, 2000; Mageed and Heikal, 2006). Change in Lake Nasser environment was occurred due to increased human activity, land reclamation, fish farming and tourist activities. In addition to the water level in Lake Nasser depends on the flood season originating from the ethiopian highlands that occurs from late August to November. The quality of water is typically determined by monitoring microbial presence, especially Faecal Coliform (FC) bacteria, which are used as microbiologic indicators for water quality. Freedom from contamination with faecal matter is the most important parameter of water quality because human faecal matter is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens (Scott *et al*., 2003).

Most nitrogen on earth is present as molecular N_2 and is biologically unavailable except for fixation by procaryotic organisms containing the enzyme nitrogenase. Since nitrate is reduced to molecular N_2 by denitrifying bacteria, fixed forms of nitrogen such as nitrate, ammonium and organic nitrogen would gradually be depleted from the biosphere, where it not for nitrogen fixation. The physiology of nitrogen-fixing organisms has long been studied and it is well known that fixation can occur in a wide variety of prokaryotic organisms, both free-living and symbiotic, including photoautotrophic cyanobacteria (blue-green algae), photoautotrophic anaerobic bacteria, aerobic and anaerobic heterotrophic bacteria and chemoautotrophic bacteria (Howarth *et al*., 1988).

The availability of fixed inorganic nitrogen often plays a fundamental role in regulating primary production in both aquatic and terrestrial ecosystems. Because, biological nitrogen fixation is an important source of nitrogen in waters, particularly if those are to be used for irrigating agricultural lands, the study of N_2-fixing microorganisms is of special concern to our understanding of global nitrogen cycle. Although a vast array of work has been done on the occurrence and activity of diazotrophs in Egyptian aquatic systems (Rabeh, 2001) and in aquatic world (Affourtit *et al*., 2001; Burns *et al*., 2002), no information is available on their contribution in Khors water microbial community. Therefore, monitoring of diazotrophs diversity in the waters sample was among the major targets of the present study. Although the importance of nitrogen fixation in aquatic ecosystems varies markedly, with planktonic nitrogen fixation contributing 82% of the total nitrogen input to various systems (Howarth *et al*., 1988), the evaluation of such bacteria on aquatic and fresh water has not been investigated so far. Hence, the present study was designed with the objective of evaluating the microbial quality and estimating diazotrophic bacteria as well as determine seasonally changes in the bacterial population.

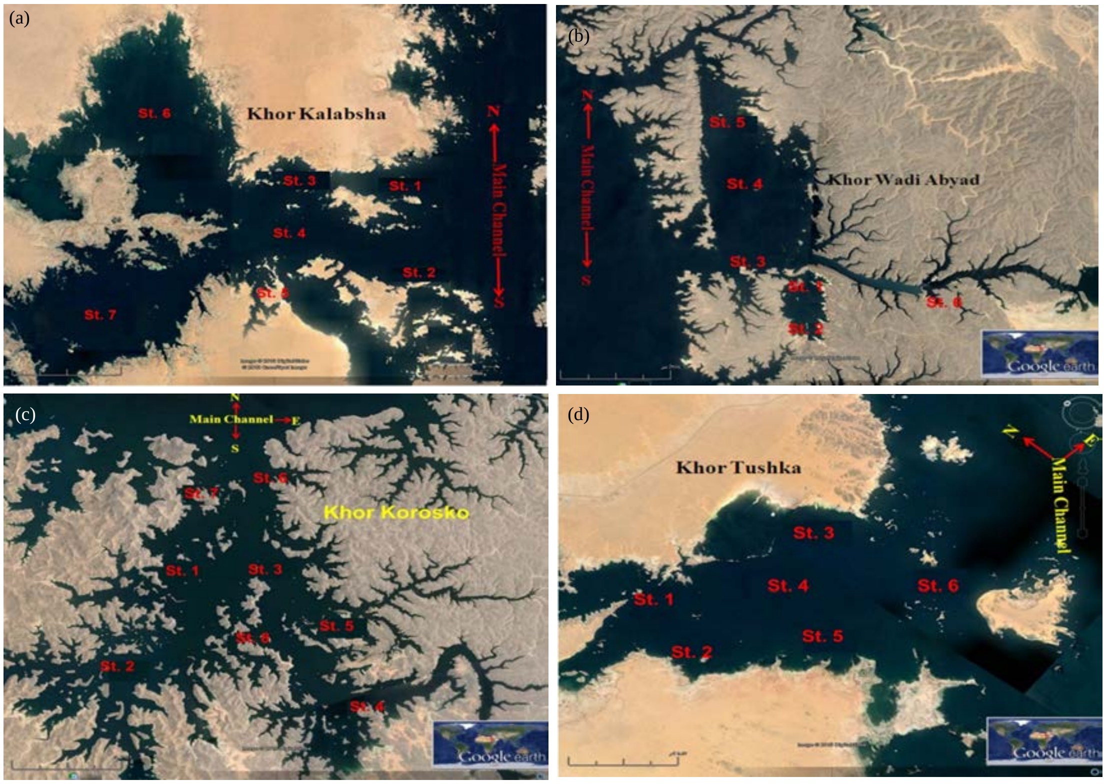
MATERIALS AND METHODS

Experimental sites: Water samples were collected from four Khors located in Lake Nasser. These Khors are Kalabsha, Wadi-Abyad, Korosko and Tushka. Khors sampling stations were illustrated in Fig. 1.

Sampling: One hundred and eighty five water samples (surface and bottom) were collected during the four seasons of the year 2013. Water samples were aseptically collected in sterile brown bottles (500 mL capacity), transported to laboratory and stored at 4°C until bacteriological analysis completed within 48 h of sampling.

Analyses: Bacteriological analysis for representative water sample was applied as follows:

- **Total bacteria:** The pour plate technique (Parkinson *et al*., 1971) and the plate count agar (APHA, 1995) were used for the enumeration of total culturable bacteria at both 22 and 37°C incubation temperatures
- **Total and faecal coliforms:** Total coliforms and faecal ones were determined by the Most Probable Number (MPN) method in Lauryl tryptose broth and EC medium (APHA, 1995)
- **Faecal streptococci:** The azide dextrose broth medium and ethyl violet aziade broth (APHA, 1995) were used for detection and counting faecal streptococci in waters
- **Total diazotrophs:** Total diazotrophs were counted using the surface inoculated plate method and N-deficient combined carbon sources agar medium, CCM (Hegazi *et al*., 1998). Three agar plates were inoculated from each suitable dilution and incubation took place at 30°C for 72 h. Representative colonies were transferred to semi-solid CCM and measured for acetylene reduction (Hegazi *et al*., 1980). Isolates producing >5 nM



(e)

Station	Kalabsha		Wadi abyad		Korosko		Tushka	
	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
1	23°38′847″	32°49′38.13″	23°21′39.62″	32°59′44.99″	22°34′25.58″	32°17′1.72″	22°37′50.12″	31°41′35.19″
2	23°31′53.85″	32°49′28.97″	23°20′55.56″	33° 0′1.03″	22°32′59.98″	32°15′35.5″	2°36′0.97″	31°40′42.09″
3	23°35′36.42″	32°45′38.88″	23°21′59.25″	32°58′53.74″	22°33′59.19″	32°18′23.37″	22°35′49.49″	31°46′21.53″
4	23°33′21.71″	32°45′2.83″	23°30′18.85″	32°58′57.78″	22°34′15.02″	32°18′52.10″	22°34′25.82″	31°44′44.18″
5	23°31′16.92″	32°45′3.21″	23°24′31.76″	32°58′25.59″	22°32′38.92″	32°19′8.13″	22°33′14.10″	31°43′17.78″
6	23°36′46.59″	32°41′57.74″	23°21′43.55″	33° 1′46.14″	22°35′27.89″	32°19′4.72″	22°32′1.5″	31°47′3.32″
7	23°30′18.85″	32°39′19.30″			22°35′28.73″	32°18′14.27″		
8					22°33′0.14″	32°17′27.13″		

Fig. 1(a-e): Satellite image location for Khors with sampling sites and GPS data, (a) Water samples stations of Khor Kalabsh, (b) Water samples stations of Khor Wadi Abyad, (c) Water samples stations of Khor Korosko, (d) Water samples stations of Khor Tushka and (e) GPS data for water samples stations

C_2H_4/culture/hour were secured for further identification based on API 20 E (Enterobacteriacea) and 20 NE (Non-Enterobacteriaceae) profiles (Othman *et al*., 2003).

Statistical analysis: Data obtained were statistically analyzed using Statistica 10 (StatSoft, Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to examine the independent effects as well as possible interactions.

RESULTS

Microbial analyses included total bacterial counts developed on either 22 or 37°C, bacteria indicators of pollution and nitrogen fixing bacteria. The ANOVA analysis indicated significant differences attributed to the seasons and the sites. Total bacteria developed on either 22 or 37°C were particularly higher in summer (up to10^6 CFU mL^{-1}) compared to other seasons, among sites the minimum count developed on either 22 or 37°C were 10^4 CFU mL^{-1} at station six in wadi Abyad. Among Khors the downstream Khors (Kalabsha and Wadi abyad) were lower than the upstream Khors (Toushka and Korosko) (Fig. 2).

Indicators of pollution were monitored in the experimental area and the obtained results are presented in (Fig. 3). The MPN of indicator bacteria ranged from 0-1100, 0-75 and 0-1100 MPN/100 mL for total coliforms, faecal coliforms and faecal streptococci, respectively. This is an indication of the suitability of the water for irrigation not for

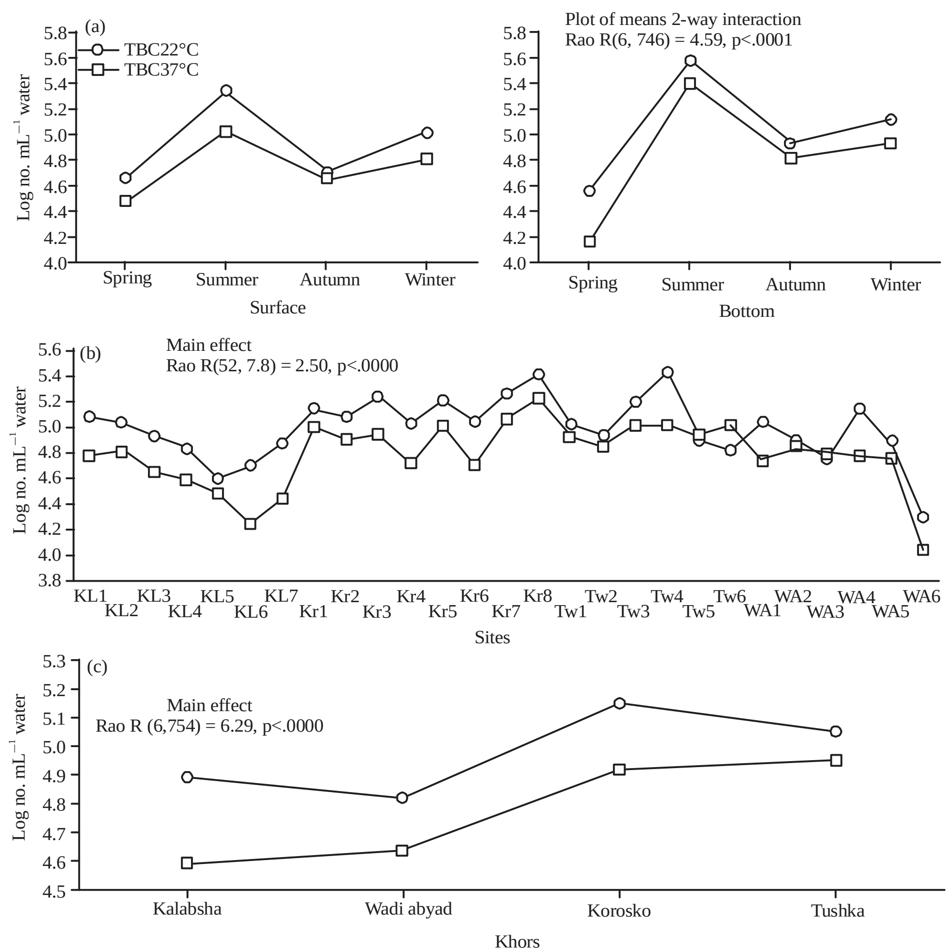


Fig. 2(a-c): Spatial and seasonal changes in microbial populations obtained along the sites of Khors during four seasons as affected by, (a) Seasons and water depth, (b) All sites of four Khors and (c) Khors (ANOVA analysis). KL1 to KL7, samples for Kalabsha Khor; Kr1-Kr 8, samples for Korosko khor; TW1-TW6, samples for Tushka khor; WA1-WA6 samples for Wadi Abyad khor (refer to Fig. 1)

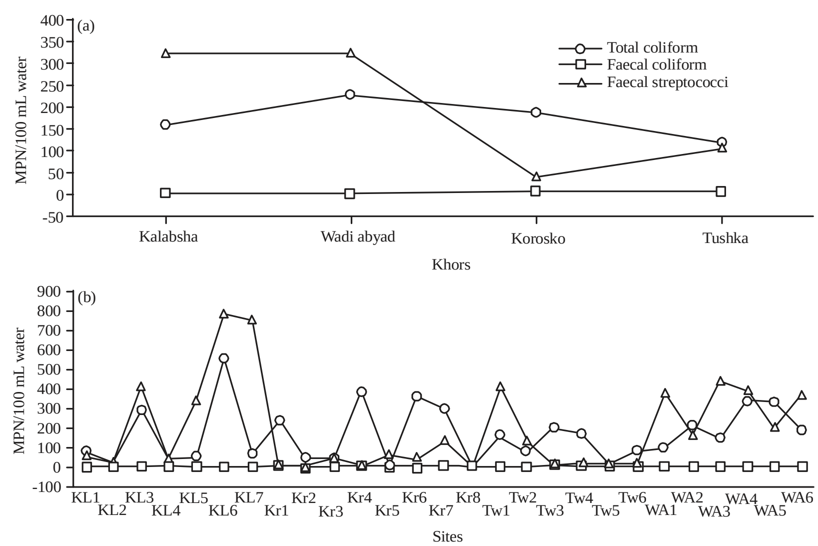


Fig. 3(a-c): Continue

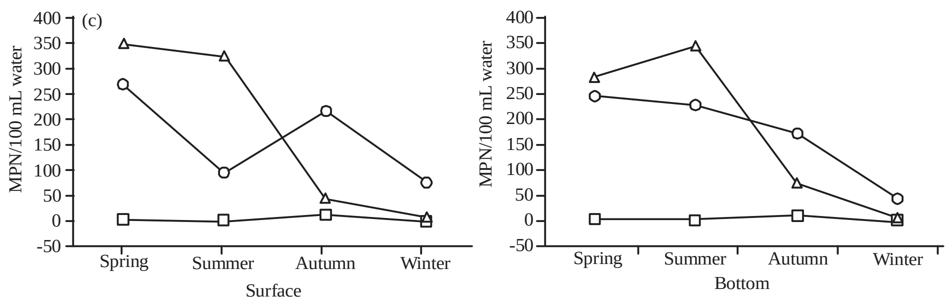


Fig. 3(a-c): Spatial and seasonal changes in the populations of bacterial indicators of pollution in water sample as affected by, (a) Khors, (b) All sites of four Khors and (c) Seasons with water depth, KL1-KL7, samples for Kalabsha khor; Kr1-Kr8, samples for Korosko khor; TW1-TW6, samples for Tushka khor; WA1-WA6 samples for Wadi Abyad khor

Table 1: FC:FS ratios calculated from samples collected at the Khors sampling stations

	winter		Spring		Summer		Autumn	
Seasons sites	surface	Bottom	surface	Bottom	surface	Bottom	surface	Bottom
Kalabsha								
KL1	0	0	0	0	0	0	**0**	**0**
KL2	0	0	0	0	0	0	**0**	**0**
KL3	0	0	0	0	0	0	**0**	**0.02**
KL4	ND	ND	0	0	0	**0.1**	**0.11**	**1.00**
KL5	ND	ND	0	0	ND	ND	**1.00**	**0.44**
KL6	ND	ND	0	0	0	0	**0.49**	**0**
KL7	ND	ND	0	0	0	0	**0.09**	**0.05**
Wadi abyad								
WA1	0	0	0	0	0	0	ND	ND
WA2	0	0	0	0	0	0	ND	ND
WA3	0	0	0	0	0	**0.02**	ND	ND
WA4	0	0	0	0	0	0	0	0
WA5	0	0	0	0	**0.02**	0	0	0
WA6	0	0	**0.01**	**0.01**		0	0	0
Korosko								
Kr1	0	0	**2.25**	**0.44**	**0.00**	**0.27**	0	0
Kr2	0	0	0		**0.44**	0	**1.00**	0
Kr3	0	0	0	**0.04**	**0**	0	**0.16**	0
Kr4	0	0	0		0	0	0	**3.57**
Kr5	0	0	0	0	0	**0.02**	0	
Kr6	0	0	0		0	**0**	0	**0.07**
Kr7	0	0	ND	ND	0	**0**	**0.11**	**0.04**
Kr8	ND	ND	ND	ND	**0**	**0**	0	0
Toushka								
Tw1	0	0	**0.01**	0	0	**0**	0	0
Tw2	0	0	0	0	**0.13**	**0**	0	0
Tw3	0	0	0	**0.60**	0	**0.13**	**3.75**	**0**
Tw4	0	0	0	**0.17**	0	**0**	0	**0.27**
Tw5	0	0	**0.09**	**0**	0	**0**	**1.00**	0
Tw6	ND	ND	**0.09**	**0**	0	0	0	0

ND: Not detected, boldface cells are those of concern

drinking. Particular enrichment of total coliforms and faecal streptococci was distinguished at the sites 6 and 7 in Kalabsha. Seasonally, water load of bacterial indicators of pollution, was lower in winter. Faecal coliforms were very rare in the majority of samples.This group of pollution indicators were absent in winter samples. In contrast, waters were rich in faecal streptococci with high numbers reaching 1100 MPN/100 mL. This group of pollution indicators were absent in few samples. The ratio of faecal coliforms to faecal streptococci ranged from 0.0-3.75 (Table 1).

The mesophilic group, diazotrophs were encountered in high population densities in all waters sampled along the experimental period. Diazotrophs were significantly lower in winter. Among the sites, site eight in Korosko recorded the

Table 2: Taxonomic position of representative isolates of diazotrophs obtained from water samples, based on API 20E and 20 NE profiles

Isolate	N_2-ase activity (nM C_2H_4/culture/hour)	API proposed taxonomic position	API profile used
KL5s A	119.67	*Klebsiella pneumonia* spp. *pneumonia*	API 20E
Kr2s sum	218	*Enterobacter sakazaki*	API 20E
Kr2B, Kr6s, Kr3s, KL3s sum, Kr5B A	199, 255, 95, 101, 83	*Klebsiella pneumoniae* spp. *pneumonia*	API 20E
KL3B sum, KL1B A	137.62, 119.67	*Enterobacter aerogenes*	API 20E
KL7B sum, Kr2s sp.	119, 71.5	*Enterobacter sakazaki*	API 20E
KL6B A	130.45	*Pseudomonas fiuorescens*	API 20NE
Tw6B A	95	*Pseudomonos putida*	API 20NE
Tw1B A	71.80	*Rhizobium radiobacter*	API 20NE
KL5B, Tw4s, KL5s, KL7 A, Kr7B sum	228, 167.5, 71, 119, 190	*Rhizobium radiobacter*	API 20NE
KL2S A	160	*Rhizobium radiobacter*	API 20NE
KL4B, WA5B, Kr8 sum, KL1B, Tw6B, Tw6B, KL7, Tw5B, Kr5 A, WA5B sp.	422, 191, 36, 301, 410, 390, 286, 190, 150, 143	*Azospirillum* spp.	ND

Sum: Summer, A: Autumn, Sp: Spring, S: Surface, B: Bottom, Kl: Kalabsha, Kr: Korosko, Tw: Toushka, WA: Wadi abyad. The taxonomic profiles were "very good identification" ND: Not determined and taxonomy based on colony and cell morphology as well as N-free cultural characteristics

Table 3: Growth and cultural characteristics based on API (20 E) profiles for diazotroph isolates

Isolates characteristics	KL5 A	Kr2 Sum	Kr2B Sum	KL3B Sum	KL7B Sum
β-galactosidase	+	+	+	+	+
Arginine dihydrolase	-	+	-	-	+
Lysine decarboxylase	-	-	+	+	-
Ornithine decarboxylase	+	+	-	+	+
Citrate utilization	-	+	+	+	+
H_2S production	+	-	-	-	-
Urease	-	+	+	-	+
Tryptophanedeaminase	-	-	-	-	-
Indole production	+	-	-	-	-
Acetoin production	-	+	+	+	+
Gelatinase	+	+	-	-	+
Fermentation/Oxidation of Glucose	0	+	+	+	+
Mannitol	+	+	+	+	+
Inositol	+	+	+	+	+
Sorbitol	+	-	+	+	+
Rhamnose	+	+	+	+	+
Sucrose	+	+	+	+	+
Melibiose	+	+	+	+	+
Amygdlin	+	+	+	+	+
Arabinose	+	+	+	+	+
Cytochrome-oxidase	-	-	-	-	-
No_2 production	+	-	+	+	+
N_2 production	-	+	-	-	-
Motility	-	-	-	+	+
MacConkey	+	+	+	+	+
Fermentation of Glucose (of-f)	+	+	+	+	+
Oxidation of Glucose (of-o)	+	+	+	+	+

+: Positive characteristics, -: Negative characteristics, according to read sheet of API manual

maximum populations of the diazotrophs. The highest values of diazotrophs were recorded for Toushka Khor (Fig. 4).

Potential isolates, having >5 nM C_2H_4/culture/hour, were identified by API 20E and API 20NE profiles (Table 2-4) being Gram negative representatives of *Klebsiella pneumonia*, *Enterobacter sakazaki*, *Enterobacter aerogenes*, *Pseudomonos fiuorescens*, *Pseudomonos putida* and *Azospirillum* sp. (Table 2-4).

DISCUSSION

Actually, the management of water quality, control of water pollution and environmental protection for preserve living conditions for the future necessitated careful routine monitoring. Therefore, the main objective of this study is to evaluate the water quality of Lake Nasser an according to the content of microbial load and diazotrophic bacteria community for some Lake Nasser Khors. Microbial analysis

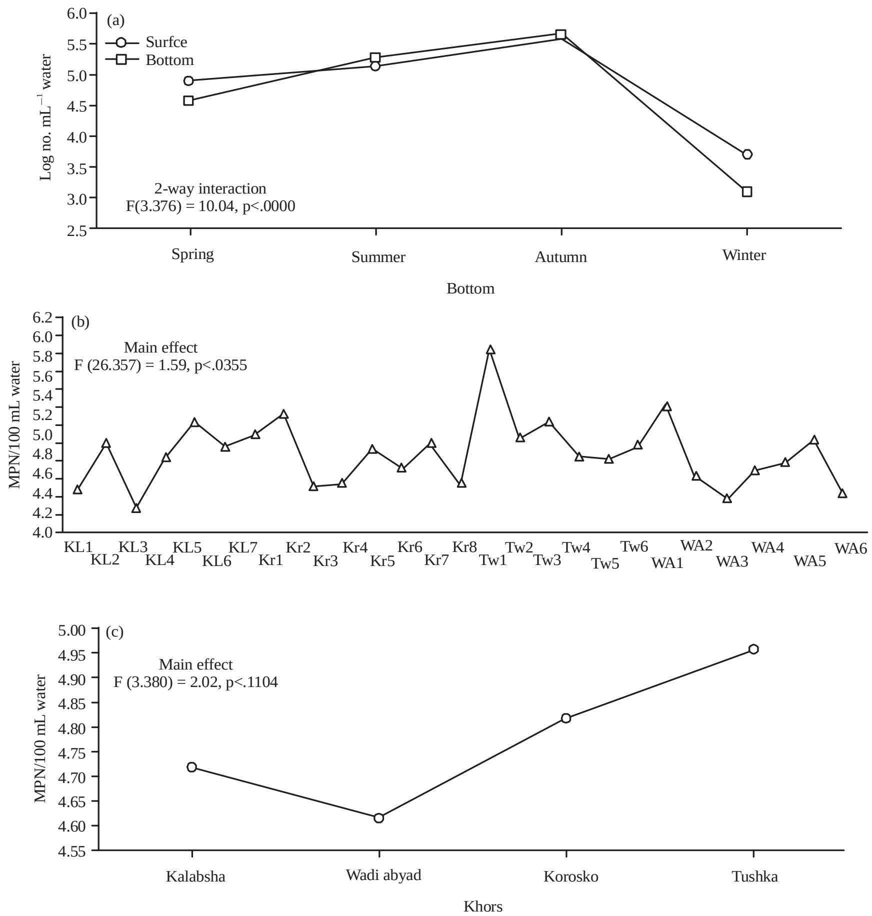


Fig. 4(a-c): Nitrogen fixing bacteria densities of water sample as affected by, (a) Seasons and depths, (b) Sites and (c) Different Khors (ANOVA analysis). KL1-KL7, samples for Kalabsha khor; Kr1-Kr8, samples for Korosko khor; TW1-TW6, samples for Tushka khor; WA1-WA6 samples for Wadi Abyad khor (refer to Fig. 1)

Table 4: Growth and cultural characteristics based on API (20 NE) profiles for diazotroph isolates

Isolates characteristics	KL6B A	Tw6B A	Tw1B A	KL5B A	KL2S A
Reduction nitrates to nitrites	+	-	-	+	-
Reduction nitrates to nitrogen	-	-	-	-	+
Indole production	-	-	-	-	-
Glucose (acidification)	-	-	-	-	-
Arginine dihydrolase	+	+	-	-	-
Urease	-	-	+	+	+
Hydrolysis (β-glucosidase)	-	-	+	+	+
Hydrolysis (protease)	-	-	-	-	-
β-galactosidase	-	-	+	+	+
Assimilation of glucose	+	+	+	+	+
Arabinose	-	+	+	+	+
Mannose	+	+	+	+	+
Mannitol	+	-	+	+	+
N-acetyl-glucosamine	+	-	+	+	+
Maltose	-	-	+	+	+
Gluconate	+	+	+	+	+
Caprate	+	+	-	-	-
Adipate	-	-	-	-	-
Malate	+	+	+	+	+
Citrate	+	+	-	-	-
Phenyl-acetate	+	+	-	-	-
Cytochrome-oxidase	+	+	+	+	+

+: Positive characteristics, -: Negative characteristics, according to read sheet of API manual

Table 5: Over all view on the analysis of Lake Nasser Khors water related to international permissible limits

Parameters	Range	Permissible limits Irrigation water	Drinking water
Total coliforms (MPN/100 mL)	0-1100	NA	0, 10 WHO
Faecal coliforms (MPN/100 mL)	0-75	unrestricted irrigation ($\leq$ or 10^3) WHO	0
Total count 22°C (CFU mL^{-1}) (authochthonous)	2×10^3-6×10^6	NA	100 EC
Total count 37°C (CFU mL^{-1}) allochthonous	1×10^3-2×10^6	NA	10 EC
Differential temperature ratio	0.88-1.32	NA	10:1 APHA

NA: Not available, boldface cells are those of concern, the superscripted values, EC (1998), WHO (2006) and APHA (1995)

indicated significant differences attributed to the seasons and the sites. Among Khors the downstream Khors (Kalabsha and Wadi abyad) were lower microbial load than the upstream Khors (Toushka and Korosko). This is due to the effect of flood and water level in Lake Nasser, where the flood is known by its high turbidity carrying a heavy load of a mixture of sand, silt and clay as well as nutrients. The yearly flood of the Nile is the most important factor affecting the conditions of lake (Mageed and Heikal, 2006). In the other side, the highest values of diazotrophs were recorded for Toushka Khors, this is may be the suitable environmental condition for growth of diazotrophs such as high organic load and low nitrogen components, also Tushka is wide and characterized by sandy bottom and slope gently.

Water load of bacterial indicators of pollution was detected in high-level comparing permissible levels of WHO (2006) and EC (1998) (Table 5). As well as the ratio of faecal coliforms to faecal streptococci ranged from 0.0-3.75, this indicating the non-human source of pollution as Geldreich and Kenner (1969) index. Where, Geldreich and Kenner (1969) suggested that the FC:FS ratio is characteristic for given types of pollution or sources of pollution. The ratio itself is not a quantitative indication of pollution but a qualitative pollution index. Thus, domestic sewage yields FC:FS ratios greater than 4.0, whereas ratios less than 0.7 indicate that the pollution derives from warm-blooded animals other than humans. The low ratios were reported in majority of sample indicating the non-human source of pollution.

Clearly noticed bacteria capable of growth on N-deficient combined carbon source medium, CCM (Hegazi *et al*., 1998) were detected in all samples collected in numbers up to 10^6, nearest the total bacteria count, this may be noticed the role of diazotrophs in aquatic environmental. Where, the role of N_2-fixer bacteria play the vital role in the aquatic environment, that essential role is not only nitrogen fixation but also rise the aquatic productivity (Tripathy *et al*., 2001; Ali *et al*., 2011). Where, they can release growth factors (Fukami *et al*., 1997; Gonzalez-Bashan *et al*., 2000) beneficial for fauna and flora (occupying intermediate position in the fish food chain) in aquatic environment as well as fish production. Moreover, the diazotrophs used successfully as probiotic for fish (Ali *et al*., 2011) and wastes bioremediators (Ali *et al*., 2012, 2014). Therefore, diazotrophs protect aquatic environment from pollution and generate value-added products, that enrich the water of Lake Nasser which is the main source of fish production in Egypt.

CONCLUSION

Results of microbiological analysis are related to the permissible levels. The Khors water is generally acceptable for irrigation and was not suitable for drinking purpose without pretreatment.

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